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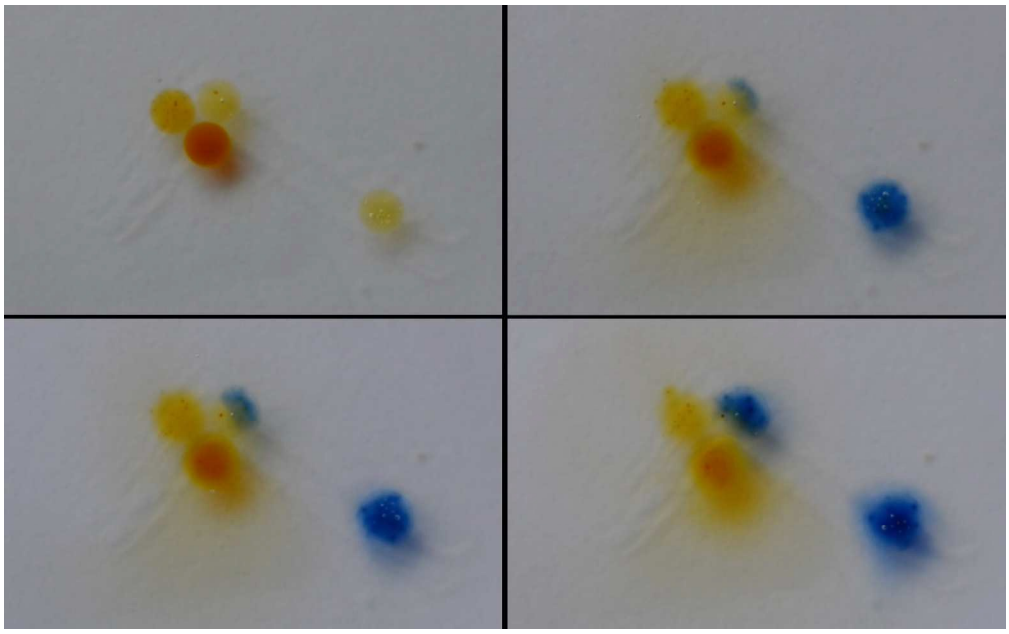

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COMMUNICATION

Communication between hydrogel beads via chemical signalling

Ross W. Jaggers and Stefan A. F. Bon *

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In this work, we demonstrate chemical communication between millimetre-sized soft hydrogel beads in an aqueous environment. Silver cations (Ag^+) and the Ag^+ chelator dithiothreitol (DTT) are used as signalling molecules. By exploiting their interplay, we conduct a series of 'conversations' between millimetre-sized beads. The communication process is monitored by tracking the response and behaviour of a central bead. This bead is loaded with the enzyme urease and has the ability to undergo a change in colour associated with a change in pH. Competitive communication between three beads, whereby the central bead receives two competing signals from two senders, is shown. We believe that our hydrogel-based system demonstrates an advance in the communication capabilities of small soft matter objects.

Communication is a significant capability of living organisms. Human beings actively communicate with each other on a physical basis, such as visually,[1] audibly,[2] or by touch.[3]

The most primitive and arguably most important form of information exchange, which also underpins the above, is chemical communication.[4,5] Living organisms rely on this method of signal transduction, such as plants,[6] vertebrates,[7] and insects,[8] as do biological cells and micro-organisms.[9]

Biological cells can communicate with one another by exchange of chemical signals, in the form of secreted signalling molecules, such as hormones, neurotransmitters or ions.[10] These molecules can be detected by receptors of a target cell that can subsequently trigger a response.[11,12] Furthermore, simple microorganisms such as the amoeba release chemical signals to induce self-assembly of individuals into multi-cellular colonies, allowing for collective motion.[13,14] It is theorised that protocells (the earliest versions of modern day cells)[15] communicated by such elementary chemical signalling, in the absence of complicated biochemical machinery.[14]

Man-made materials have been proposed and designed in an effort to mimic communication in and between organisms. The ability to exchanges information by means of chemical signal transduction has been demonstrated computationally, for example gels and microcapsules that undergo communication and auto-chemotaxis,[14,16–19] vesicles that communicate through nanotubes,[20] and microcapsules that self-assemble.[21]

A number of interesting studies have demonstrated that this kind of communication between synthetic objects is indeed realistic. Sen and co-workers showed that silica particles flock around photo-active silver chloride colloids in water, which themselves show schooling behaviour. The swarming phenomenon is induced by localised electrolyte gradients established through secretion of ions.[22] Giménez *et al.* designed capped silica nanoparticles that can sent hierarchical messages, such that a chemical signal opens a gate that allows for a secondary signal to be sent.[23] Furthermore, communication mediated cargo delivery, where Janus nanoparticles must interchange chemical messengers in order to open a series of gates, has been demonstrated.[4]

Moving away from nanoscale dimensions, communication between an interconnected network of giant vesicles has been demonstrated by Orwar and coworkers.[24,25] The lumens of vesicles, connected by nanotubes, are able to exchange material by their interconnecting bridges. Recently, Mansy *et al.* elegantly designed artificial cells which have the ability to sense and synthesize quorum signalling molecules, allowing for chemical communication with a range of bacteria.[26]

At macroscopic length scale, the oscillatory, non-equilibrium Belousov–Zhabotinsky (BZ) reaction has been used to propagate chemical waves across a series of hydrogel objects, thus enabling them to pass on a signal.[27,28]

In this work, we demonstrate that millimetre-sized, soft hydrogel objects containing different signalling and receiving molecules, can communicate by exchange of chemical signals

Department of Chemistry, University of Warwick, Coventry, C47 7AL, UK.
E-mail: S.Bon@warwick.ac.uk; Web: www.bonlab.info; Tel: +44 (0)2476 574009

Electronic Supplementary Information (ESI) available, as well as supporting videos from which figures 2 – 5 are taken. See DOI: 10.1039/x0xx00000x

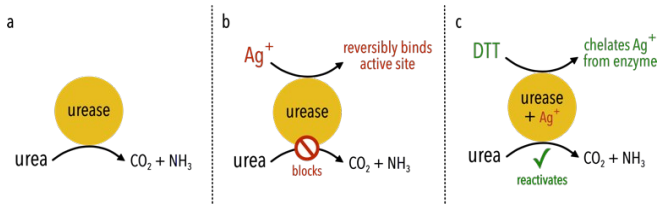

Figure 1 The three modes of action of a urease loaded hydrogel bead (a) when exposed to an acidic aqueous solution of urea, a bead containing urease generates carbon dioxide and ammonia within its core and in its surrounding environment (b) if a 'urease' bead is exposed to aqueous silver ions (Ag^+), the ions reversibly bind to the enzyme active site and inhibit it. The bead can no longer produce carbon dioxide or ammonia (c) if a silver bound 'urease' bead is exposed to dithiothreitol (DTT), Ag^+ ions can be chelated from the enzyme and thus reactivate it, allowing it to produce carbon dioxide and ammonia once again.

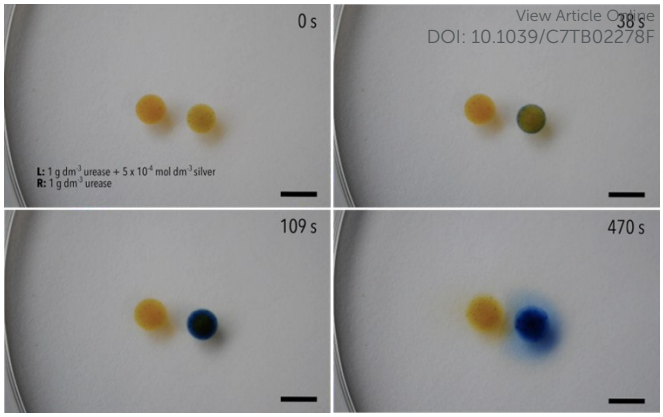

Figure 2 Two beads are immersed in a 0.1 mol dm^{-3} solution of urea; the left bead contains urease at a concentration of 1 g dm^{-3} and silver nitrate at a concentration of 5 x 10^{-4} mol dm^{-3}, and the right contains urease at a concentration of 1 g dm^{-3} only. After 25 seconds, the bead containing only urease increases in pH past a value of 7.6, signified by a change in colour of the pH indicator bromothymol blue from yellow to blue. The bead containing Ag^+ ions does not undergo this pH and colour change. Scale bar = 5 mm. See supporting video for full sequence.

when placed in proximity to each other in an aqueous environment. Simple chemical and biological tools are used to allow for communication to operate in short timescales (seconds to minutes, see figure 1). Beads encapsulating one of three species, namely the enzyme urease, the enzyme inhibitor silver (Ag^+), and the Ag^+ chelator dithiothreitol (DTT), are shown to interact when placed in contact with one another. By exploiting the interplay between the enzyme, its reversible inhibitor, or this inhibitor's chelator, we demonstrate a series of 'conversations' between the beads, following the release of encapsulated Ag^+ and DTT into the surrounding solution and adjacent beads.

These communicating beads are an original example of chemically mediated signalling between mixed soft artificial objects, and provides insight into the design of biomimetic materials that are able to communicate without continuous external input.

Hydrogel beads are formed from aqueous solutions of the biopolymer sodium alginate, and are formed by depositing the solution from a pipette tip into a 0.1 mol dm^{-3} aqueous solution of calcium chloride hexahydrate or calcium hydroxide. The desired components, either urease, silver cations or DTT, are dissolved into the alginate solution prior to deposition, along with the colorimetric pH indicator bromothymol blue. Acetic acid is used to adjust the pH to 3.5 for both the alginate and the calcium ion solutions. The beads reside in the calcium solutions for only a few minutes, resulting in a solid cross-linked shell and a liquid core (see supporting information for the full experimental protocol).

As a monitoring tool in our study we focus on the colorimetric behaviour of an 'enzyme' bead. This alginate hydrogel bead is loaded with the enzyme urease at a concentration of 1 g dm^{-3}, which is entrapped in the bead and cannot leave.[29,30] Upon exposure to a 0.1 mol dm^{-3} aqueous solution of urea (also corrected to pH 3.5), the urease generates carbon dioxide and ammonia, thus increasing the pH in the bead and its immediate local area (see figure 1a). Inclusion of the colorimetric pH indicator bromothymol blue within the bead allows us to visualise this change that manifests as a transition from yellow to dark blue when a pH of 7.6 is surpassed (see figure 2, right bead). This colour change happens after a defined time period, in this case after 25 s. Note that the dormancy period can be tailored by variation of the enzyme concentration. A lower concentration of urease results in an increased delay before pH increase, and thus colour change from yellow to blue thanks to the bell-shaped pH activity curve for this enzyme (see our previous study for further information about this time programming, as well as work by Walther *et al.*).[31–33]

The first communication tool we employ is the deactivation of urease using silver ions (Ag^+).[34]

Silver (as well as other heavy metal) ions are able to reversibly bind with the active site of urease, deactivating it, and thus halting its production of carbon dioxide and ammonia (see figure 1b).[35] This means that a urease containing bead introduced to silver ions, in the form of an aqueous silver nitrate solution, is unable to undergo a pH increase when exposed to a solution of urea. We checked this by preparing a bead which contained both urease at a concentration of 1 g dm^{-3} (identical to its active partner beside it) and silver ions at a concentration of 5 x 10^{-4} mol dm^{-3}. Its behaviour is compared with the 'enzyme' bead (see Figure 2). The presence of silver ions in the left bead halts the production of ammonia and prevents a colour change, thus acting as an 'off' switch.

This interplay between urease and silver can be used as a tool for communication between two gel beads. If a bead containing urease sits next to a bead containing silver nitrate, silver ions diffuse from the 'silver' bead into the 'enzyme' bead. This will lead to local deactivation of urease and thus the switching off of the colour change. Figure 3 displays three scenarios a, b, and c, in which the concentration of entrapped

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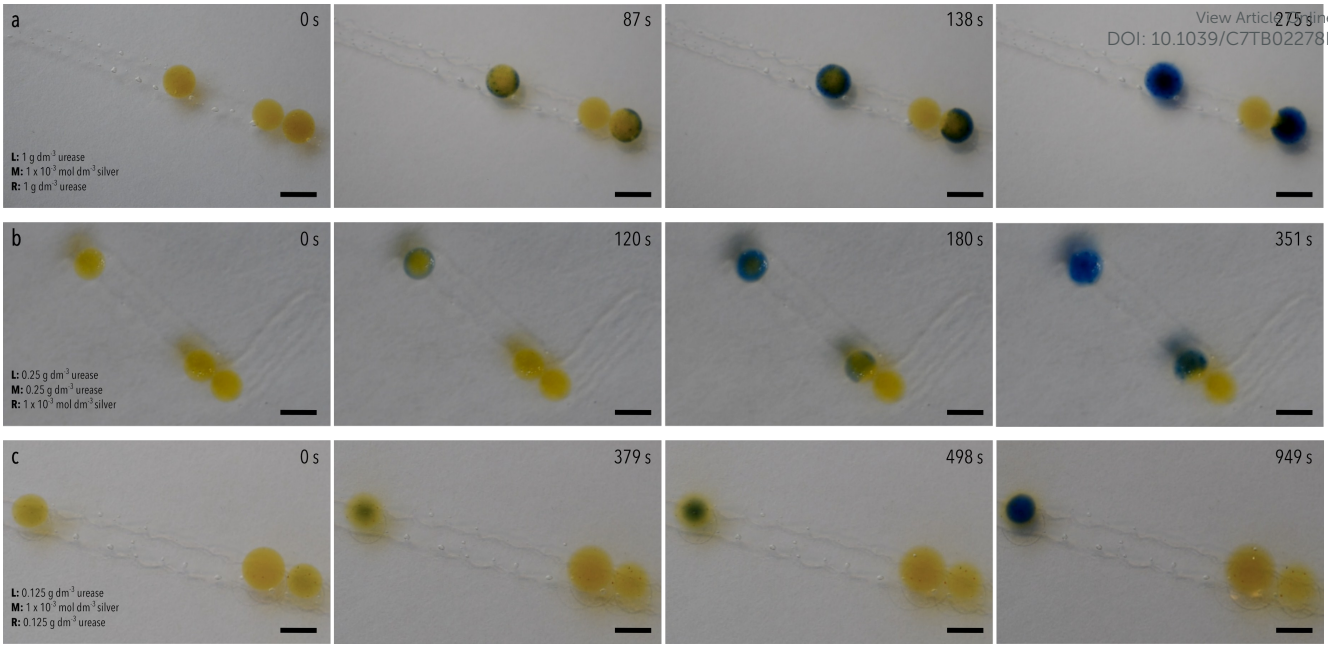

Figure 3 When a urease containing bead is placed next to a bead containing 1 x 10^{-3} mol dm^{-3} silver ions in a 0.1 mol dm^{-3} solution of urea, it is either partially or fully inhibited, depending on the concentration of urease in the bead (a) In the case of an enzyme concentration of 1 g dm^{-3}, contact with the 'silver' bead prevents a small region of the bead from increasing in pH and thus it remains yellow, this region being the contact point between the 2 beads. The rest of the bead is, however, able to increase in pH in the same fashion as its uninfluenced neighbour to the left (the onset of colour change to blue occurring at 38 seconds) (b) When the enzyme concentration in the beads is lowered to 0.25 g dm^{-3}, a similar behaviour is observed, however the yellow region at the contact point is widened, such that a large portion of the enzyme bead remains at low pH after 351 seconds. The uninfluenced bead transitions to high pH and blue at 82 seconds (c) When the enzyme concentration is lowered further to 0.125 g dm^{-3}, this yellow region is extended fully across the entire 'enzyme' bead, remaining after 949 seconds. The uninfluenced bead transitions to high pH and blue at 218 seconds. Scale bar = 5 mm. See supporting video for full sequence.

enzyme is varied, this being 1, 0.25 and 0.125 g dm^{-3}, respectively. Each experiment contains three beads aligned. In scenarios a and c, the outer two (left and right) are 'enzyme' beads and the middle bead contains silver at a concentration of 1 x 10^{-3} mol dm^{-3}. This 'silver' bead is placed in direct contact with the right hand 'enzyme' bead. In scenario b, the left and middle beads are 'enzyme' beads, whilst the right-hand bead is 'silver'.

In each case, the bead on the left transitions from yellow to blue indicating an increase in pH, and the time taken for this to happen increases with a decreasing enzyme concentration. For a 1 g dm^{-3} bead, the onset of colour change is at 38 seconds, for a 0.25 g dm^{-3} bead it is at 82 seconds, and for a 0.125 g dm^{-3} bead it is at 218 seconds. The right 'enzyme' bead, however, displays a different behaviour, on account of its interaction with the 'silver' bead.

In the case of an enzyme concentration of 1 g dm^{-3} (top row, scenario a, figure 3), contact with the 'silver' bead deactivates a small region of the 'enzyme' bead hereby arresting its colour change. This region is in proximity to the contact point between the two beads. The majority of the bead remains enzymatically active, however, and thus able to undergo the colour transition from yellow to blue.

When the enzyme concentration in the beads is lowered to 0.25 g dm^{-3} (middle row, scenario b, figure 3) a similar behaviour is observed. Note that we exchanged the two beads, so now the 'silver' bead is on the outer right. The yellow region at the contact point is widened, such that a larger portion of the 'enzyme' bead remains at low pH (and thus yellow) after 351 seconds.

When the enzyme concentration is lowered further to 0.125 g dm^{-3} (lower row, scenario c, figure 3), this yellow region is extended fully across the entire 'enzyme' bead, and remains yellow after 949 seconds.

From these observations, we deduce that the silver ions loaded into 'silver' bead are able to diffuse into the 'enzyme' bead with which they make contact but not with the 'enzyme' bead found further afield. The different responses of the 'enzyme' beads to this influx of silver ions is explained by the time-delayed behaviour of the urease.

The concentration of silver used, here 1 x 10^{-3} mol dm^{-3}, far exceeds that of the amount needed to deactivate each urease molecule. In scenario a of figure 3, *ca.* 1.8 x 10^{-6} mol dm^{-3} of urease was present (at 545 kDa). We therefore postulate that the enzymatic reaction at higher enzyme loadings generates a sufficiently high flux to influence the diffusional flow profile of the silver ions, hindering bead penetration. When the enzyme

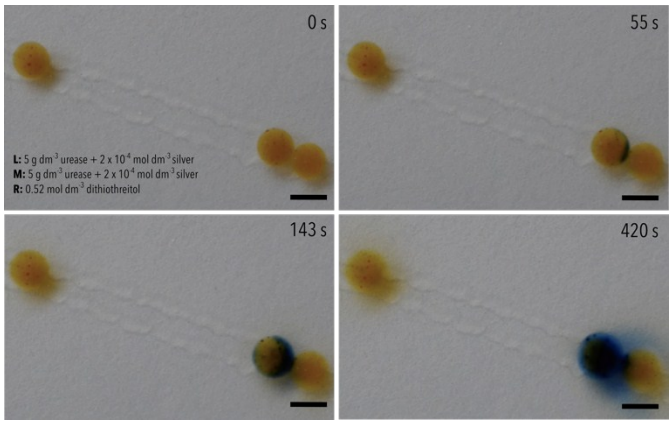

Figure 4 When a bead containing both urease, at a concentration of 5 g dm^{-3}, and silver, at a concentration of 2 x 10^{-4} mol dm^{-3}, is immersed in a 0.1 mol dm^{-3} solution of urea, no pH increase is observed (left bead). If an identical bead (middle bead) makes contact with one containing 0.52 mol dm^{-3} DTT (right bead), the contained DTT diffuses into the silver-bound 'enzyme' bead. This results in the chelation of silver ions from the urease and thus its reactivation. Scale bar = 5 mm. See supporting video for full sequence.

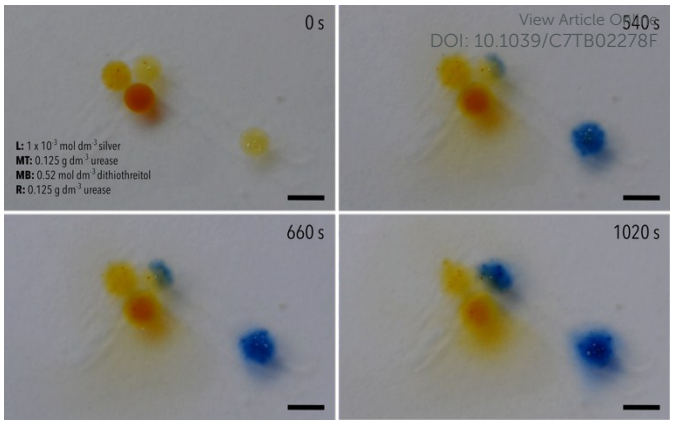

Figure 5 Just as in figure 3 c, a 1 x 10^{-3} mol dm^{-3} 'silver' bead sits next to a 0.125 g dm^{-3} 'urease' bead (far left and the top middle bead, respectively) in an aqueous solution of 0.1 mol dm^{-3} urea. In this case, however, a third bead is introduced, namely a 0.52 mol dm^{-3} 'DTT' bead (bottom middle). The far-right bead, containing 0.125 g dm^{-3} urease, undergoes its colour and pH change as expected (onset at 214 seconds). As the left-hand 'enzyme' bead makes contact with a 'silver' bead, a pH increase after this time period is not observed, as the silver binds to the enzyme active site. After a longer delay (420 seconds), a pH increase is observed, in contrast to figure 3 c where no 'DTT' bead is present. Scale bar = 5 mm. See supporting video for full sequence.

concentration is low, this counter flux caused by the enzymatic reaction is too low to stop complete penetration of the silver ions, hereby deactivating the 'enzyme' bead fully.

The second communication tool we employ is the reactivation of urease by chelating the bound silver ions with dithiothreitol (DTT), see figure 1 c.[36,37]

When a bead containing both urease, at a concentration of 5 g dm^{-3}, and silver cations, at a concentration of 2 x 10^{-4} mol dm^{-3}, is immersed in a 0.1 mol dm^{-3} solution of urea, no pH increase is observed (left bead in figure 4, confirming figure 2). If an identical bead makes contact with one containing 0.52 mol dm^{-3} DTT, the encapsulated DTT diffuses into the silver-deactivated 'enzyme' bead. This results in the chelation of silver ions from the urease and thus its reactivation. This is visualised on the right-hand side of figure 4, where the 'DTT' bead (far-right) makes contact with a silver-bound 'urease' bead (centre), inducing a transition from yellow to blue. This quite clearly shows that DTT can act as an 'on' switch to a previously deactivated 'urease' bead, and that the 'urease' bead receives a chemical signal from the 'DTT' bead.

It is worth noting that the two-step modification of urease activity, by means of silver cations (Ag^+) followed by dithiothreitol (DTT), can be used reversibly, as demonstrated in the sequential starting and stopping of swimming particles by Sánchez *et al.*[38] Addition of Ag^+ inhibits the enzyme, addition of DTT removes the inhibitor, and thus addition of more Ag^+ re-inhibits the enzyme, *etc*. For as long as DTT is at a molar excess of 120:1, silver is chelated. Any lower than this, and free silver is present that binds to urease.

In the context of our colorimetrically visualised communication, reversible action is not so easily demonstrated. Enzyme action produced an increase in pH that results in a dark blue colour. Though urease inhibition is quick, a colorimetric change back to yellow is slow, as generated ammonia in the bead must exchange with the surrounding bulk acidic solution. We observe this exchange over a period of *ca.* 20-30 minutes, by which time the beads have also lost their pH indicating dye. For this reason, reversible action in the confines of our experimental set-up is not explored.

In the final communication scenario shown in figure 5, our two switches are used in unison to create a trio of communicating beads.

Just as in figure 3 c, a 1 x 10^{-3} mol dm^{-3} 'silver' bead sits next to a 0.125 g dm^{-3} 'urease' bead (far left and the top middle bead, respectively). In this case, however, a third bead is introduced, namely a 0.52 mol dm^{-3} 'DTT' bead (bottom middle). The far-right bead, containing 0.125 g dm^{-3} urease, undergoes its colour and pH change as expected (onset at 214 seconds).

As the left-hand 'enzyme' bead makes contact with a 'silver' bead, a pH increase after this time period is not observed, as the silver inhibits the enzyme. After a longer delay (420 seconds), a pH increase is observed, in contrast to figure 3 c where no 'DTT' bead is present. We infer that the 'DTT' bead present in figure 5 competes with the 'silver' bead, chelating the silver removing any silver blocking urease activity. Importantly, the observed behaviour of the 'enzyme' bead requires communication with both the 'silver' and 'DTT' bead, in the form of two competitive chemical signals.

Conclusions

In conclusion, we have demonstrated that by using a trio of simple biochemical, inorganic and organic molecules, we can

conduct exchanges of communications, or 'conversations' between soft hydrogel beads. The interplay between the enzyme urease, the enzyme inhibitor silver (Ag^+), and the Ag^+ chelator dithiothreitol (DTT) allows for exchange of information of complexity analogous to signalling in simple microorganisms and to the physiochemical mechanisms theorised to have been used by the earliest protocells. We believe that this system demonstrates an advance in the communication capabilities of entirely soft systems. Briefly considering the applications of this work, if reversible action of Ag^+ and DTT is achieved, repetitive communication could be established. For example, beads could be sequentially turned 'off' and 'on' a number of times, generating a system that resembles more of a sensor. Such a system would need a larger continuous sink, flow-cycled so as to remove excess Ag^+ and DTT.

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